TUBERIZATION AND TUBER DORMANCY IN *Hydrilla verticillata* (L.f.) ROYLE

By

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To my wife Virdell

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	viii
LIST OF FIGURES	x
ABSTRACT	xii
CHAPTER I. GENERAL INTRODUCTION AND LITERATURE REVIEW.	1
Introduction	1
Biology of Hydrilla Shoots and Propagules	4
Literature Review	12
CHAPTER II. GENERAL METHODS AND PROCEDURES	21
Introduction	21
Harvesting Hydrilla Tubers	21
Determination of Mineral Composition of Tubers	24
Sprouting Tubers under Several Temperature Regimes	24
Sprouting Tubers under Different Atmospheric Compositions	25
Determination of Respiration of Mature Hydrilla Tubers	25
Drying Hydrilla Tubers	26
Determination of the Effect of Light on Hydrilla Tuber Sprouting	26
Nitrogen Determination	26
Determination of Sucrose and Reducing Sugars	29
Determination of Starch	31
Application of Herbicides and Growth Regulators	32
CHAPTER III. CHARACTERISTICS OF HYDRILLA TUBERS HARVESTED AT RODMAN RESERVOIR	34
Introduction	34
Materials and Methods	34
Results and Discussion	35
The Location of Tuber Formation	35
Some Characteristics of Tubers	35
Mineral Nutrient, Carbohydrate, Nitrogen, and Lipid Composition of Tubers	37

Page

The Sprouting of Tubers under Continuous Light or Darkness. 41
The Meristem of the Tuber 41
The Effect of Water Drawdown on Hydrilla Tuber Sprouting. 42
Variability in the Quantity of Tubers from One Small Area to Another. 42

CHAPTER IV. EFFECT OF WATER DEPTH ON HYDRILLA TUBERS COLLECTED FROM RODMAN RESERVOIR DURING DRAWDOWN 44
Introduction 44
Materials and Methods. 44
Results and Discussion 46
Effect of Water Column Depth on the Size, Distribution, and Sprouting of Tubers. . 46
Sprouting of Hydrilla Tubers under Continuous Light and Dark Conditions. 46
Quantity of Hydrilla Tubers/m^2. 48
Effects of Light Quality on Hydrilla Tuber Sprouting. 48

CHAPTER V. EFFECTS OF TEMPERATURE ON HYDRILLA TUBER SPROUTING 52
Introduction 52
Materials and Methods. 52
Results and Discussion 54
Effects of Temperature on Hydrilla Tuber Sprouting. 54
Optimal Temperature for Hydrilla Tuber Sprouting. 54
Low Temperature Inhibition of Hydrilla Tuber Sprouting. 58

CHAPTER VI. EFFECTS OF DIFFERENT STORAGE REGIMES ON HYDRILLA TUBER SPROUTING 65
Introduction 65
Materials and Methods. 65
Results and Discussion 66
Storage of Tubers under 100% Nitrogen 66
Storage of Hydrilla Tubers under Nitrogen, Oxygen, and Carbon Dioxide 67
Storage of Hydrilla Tubers in Several Concentrations of Carbon Dioxide. 69
Storage of Hydrilla Tubers in Moist Peat for 4, 5, 7, and 8 Weeks 71
Storage of Hydrilla Tubers in Moist Peat for 6, 15, 30, 32, or 35 Weeks 73

CHAPTER VII. EFFECTS OF DRYING ON HYDRILLA TUBER SPROUTING 76
Introduction 76
Materials and Methods. 76
Results and Discussion 77

Page

CHAPTER VIII. EFFECTS OF SEVERAL GROWTH REGULATORS AND HERBICIDES ON THE SPROUTING OF HYDRILLA TUBERS. 81
Introduction 81
Materials and Methods. 81
Results and Discussion 82
Banvel. 82
Eptam 82
Ethrel. 84
Endothall 85
Effects of Several Growth Regulators on Hydrilla Sprouting 85

CHAPTER IX. SUMMARY AND CONCLUSIONS. 88

REFERENCES. 92

BIOGRAPHICAL SKETCH 98

LIST OF TABLES

		Page
Table 1.	Some characteristics of hydrilla tubers harvested at Rodman Reservoir during January	36
Table 2.	Mineral content of hydrilla tubers	38
Table 3.	Gross organic chemical composition of hydrilla tubers	39
Table 4.	Analysis of water in which hydrilla tubers were actively being formed	40
Table 5.	Light and dark sprouting, fresh weight, and distribution of hydrilla tubers taken from various depths	47
Table 6.	Effect of different light regimes on hydrilla tuber sprouting	49
Table 7.	Light stimulation of hydrilla tuber sprouting under aerobic or anaerobic conditions	51
Table 8.	Effects of temperature on hydrilla tuber sprouting	57
Table 9.	Reversal of low-temperature inhibition of sprouting of hydrilla tubers	61
Table 10.	Effect of light and atmospheric gases on sprouting of hydrilla tubers and turions	68
Table 11.	The effect of different storage periods in carbon dioxide and nitrogen on hydrilla tuber sprouting	70
Table 12.	The effects of time of harvest on the sprouting of hydrilla tubers	72
Table 13.	The effect of storage in peat on hydrilla tuber sprouting	75
Table 14.	Sprouting and respiration of hydrilla tubers after several drying periods	80

LIST OF TABLES (contd.)

Page

Table 15. Some effects of herbicides on hydrilla tuber sprouting and shoot length. 83

Table 16. Some effects of growth regulators on hydrilla tuber sprouting and meristem length. 86

LIST OF FIGURES

		Page
Figure 1.	Vegetative portion of hydrilla (*Hydrilla verticillata* [L.f.] Royle) showing shoots and roots extending from the stolon.	6
Figure 2.	Apical meristem of hydrilla (*Hydrilla verticillata* [L.f.] Royle) showing the vascular strands and scale-like leaves that surround the meristem (25X)	9
Figure 3.	Tuber of hydrilla (*Hydrilla verticillata* [L.f.] Royle) (25X).	11
Figure 4.	Map of Rodman Reservoir (Lake Ocklawaha) at eighteen feet mean sea level. Asterisk indicates area where *Hydrilla verticillata* (L.f.) Royle tubers were collected (22). . .	23
Figure 5.	The transmission of light through red, green, and blue filters. Open circle is blue filter, filled square is red filter, and filled circle is green filter.	28
Figure 6.	*Hydrilla verticillata* (L.f.) Royle tubers that were sprouted on a temperature-gradient bar under continuous light conditions. The dotted line indicates tubers which had low temperature inhibition of sprouting reversed when the tubers were placed in a germinator under continuous light at a constant temperature of 30 C. . .	56
Figure 7.	*Hydrilla verticillata* (L.f.) Royle tubers that were sprouted on a temperature-gradient bar under continuous dark conditions. The dotted line indicates tubers which had low temperature inhibition of sprouting reversed when the tubers were placed in a germinator under continuous light at a constant temperature of 30 C	60

LIST OF FIGURES (Contd.)

Page

Figure 8. *Hydrilla verticillata* (L.f.) Royle turions that were sprouted on a temperature-gradient bar under continuous dark conditions. The dotted line indicates turions which had low temperature inhibition of sprouting reversed when the turions were placed in a germinator under continuous light at a constant temperature of 30 C. 64

Figure 9. Sprouting of *Hydrilla verticillata* (L.f.) Royle tubers after several drying periods. . 79

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Hydrilla verticillata (L.f.) Royle tubers were harvested from a shallow water reservoir during drawdown. Experimental results and field observations indicated that dormant tubers were present in the reservoir during the entire year. The dormant tubers were found approximately 0.08 to 0.1 m below the surface of the hydrosoil. After the water level was raised, tubers found at water column depths of 0.3 and 0.6 m started to sprout during the summer months; however, dormant tubers were still abundant at deeper depths.

The quantity and size of tubers in a particular area varied depending upon the depth of the water column. Variation in the number of tubers found within a small area also was extremely large. Hydrilla tubers showed an increase in tuber size with increase in depth of water column from 0.3 to 1.5 m. The number of tubers/m^2 increased with increases in water column depth from 0.3 to 0.9 m.

Tubers remained dormant for more than 9 months when they were stored in moist peat in darkness at a temperature

of 25 C. Tubers placed under 100% nitrogen and 100% oxygen during a 14-day treatment at a temperature of 30 C had sprouting percentages that were higher under continuous light than under continuous dark. Tubers stored under 100% carbon dioxide exhibited no sprouting under either continuous light or dark conditions. Tubers sprouted under continuous light consistently had higher sprouting percentages than tubers sprouted in the dark. This was true regardless of whether the conditions were aerobic or anaerobic.

At low temperatures, hydrilla tubers were inhibited from sprouting. This inhibition was reversed when the tubers were placed subsequently in a germinator under continuous light at a temperature of 30 C. The optimum sprouting temperature for hydrilla tubers was between 18 C and 32 C in darkness and 26 C under light conditions. Tubers that were dehydrated from 1 to 58 h had a decrease in sprouting with increase in dehydration. Tubers dried for 32, 44, and 58 h exhibited no sprouting even after complete rehydration had occurred.

The herbicides banvel (3,6-dichloro-o-anisic acid), eptam (S-ethyl dipropylthiocarbamate), ethrel (2-chloroethylphosphonic acid) and endothall (the mono[N,N-dimethylalkylamine]salt of 7-oxabicyclo[2,2,1]heptane-2,3 dicarboxylic acid) applied at four concentrations had no effect on the sprouting of hydrilla tubers.

Gibberellic acid, napthaleneacetic acid, and thiourea had no effect on the sprouting of hydrilla tubers. Tubers that were sprouted in 500 mg/l maleic hydrazide had stunted shoots and exhibited only 73% sprouting.

CHAPTER I
GENERAL INTRODUCTION AND LITERATURE REVIEW

Introduction

The subterranean propagules of hydrilla were first reported by MacKenzie in Dade County, Florida in 1967 (34). These propagules were found during the course of residue sampling in the bottom soil of canals. The tubers were quickly recognized as a rapid source of reinfestation of hydrilla after a herbicide application. Since tubers are a threat to hydrilla control, investigation of conditions under which the tubers remain viable is in order.

Research on hydrilla propagules has been almost non-existent except for work done by Mitra (42), and Steward (54) and Sutton[1], both of the University of Florida Aquatics Laboratory in Fort Lauderdale, Florida. Efforts to combat the spread of hydrilla are fruitless unless some control is available to regulate tuber formation and tuber sprouting of hydrilla *in situ*. Efforts should also be made to educate the general public concerning transportation of noxious weeds from one body of water to another. Weber (64) reported that dormancy is strongest when the turions abscise from the parent plant and is affected by both day length and temperature. Delay of sprouting is a problem arising from the great varia-

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[1]Personal communications, Dr. David L. Sutton, 1973, University of Florida Agricultural Research Center, Fort Lauderdale, Florida.

tion in metabolic activity among tubers. Variation in sprouting is prevalent not only *in situ* but under controlled conditions in the laboratory. Knowledge of the physiology and factors affecting the development of hydrilla tubers is essential if any method of control is going to be successful. Whether the control is biological or chemical or a combination of both is dictated by the particular situation at hand.

In 1948, E. West, a botanist for the Florida Agricultural Experiment Station at Gainesville, said, "In general, the habits of plants as groups seem more important than the botanical characteristics of the species. As the tolerance or immunity to specific chemicals becomes more apparent, certain species may become more important or demand special treatment" (65, p. 18). At the time this statement was made, a tropical plant known as *Hydrilla verticillata* (L.f.) Royle was not mentioned as a significant noxious aquatic macrophyte. The plant had not been introduced into this country. In 1975, this plant has become very important and demands special treatment since, in a span of only 15 to 20 years, it has begun to dominate bodies of water over the entire state of Florida and is rapidly spreading to other states (17).

Hydrilla has become a significant threat to aquatic resources and as the use of waterways intensifies, the problem this plant pest presents becomes even more significant. Some of the problems already realized include: (1) the obstruction of water flow and proper drainage, which could cause flooding of untold acreage and loss of lives and crops in flood control

districts; (2) interference with navigation and prevention of fishing and recreation; (3) the lowering of real estate values; and (4) the creation of a health hazard by providing breeding places for mosquitoes, snakes, and other organisms (6,23).

Since being introduced into the United States in the 1950's as an aquarium plant, hydrilla has completely dominated the entire bottom growth of thousands of acres of fresh water resources (17). Submersed macrophytes such as hydrilla are difficult to control by herbicides or machines. Herbicide treatment must be done to the entire volume of water which may lead to fish kill and death of beneficial aquatic organisms (7).

Hydrilla forms a dense canopy at the water's surface and limits light penetration by at least 95% in the top 30.0 cm of water (17). The low ratio of root tissue to foliage produced by hydrilla allows that the majority of fixed carbon to be used in the production of the competitive standing crop. The production of many subterranean propagules/ha and the presence of large numbers of axillary meristems on hydrilla favors colonization and makes control difficult (17).

Hydrilla is found in still or slow-moving fresh and brackish waters at depths of 1 to 20 m (6,34). Subterranean propagules are formed by hydrilla over this entire range of depths, and these are considered the primary mode of reinfestation because of their dormancy and inaccessibility to various types of herbicides.

In early studies, hydrilla propagules were called turions (40,41,42,43). These turions were classified as being underground or floating. The underground turions developed terminally on positive geotropic shoot tips of axillary branches. The branches are generally long and near the mud level (Figure 1). The green turion develops in the axils of leaves above the ground and has a short stalk. The underground turions are generally larger in size than the above-the-ground turions. When fully formed, the green turions drop to the hydrosoil. The underground turion also becomes detached from the parent plant. When turions sprout, they are generally characterized by shoots with short internodes bearing green whorled leaves and roots. Rarely does more than one shoot form from a single turion. Once sprouting begins, it takes 12 to 14 days for formation of a fully developed plant. The turions are present December through January after which they abscise and are found in the hydrosoil from April onwards. Large-scale shedding of turions from parent plants has been observed from May to the end of June (42). In this paper, turion will refer to propagules produced in the axil of leaves, and the term tuber will refer to the propagules produced on underground stolons.

Biology of Hydrilla Shoots and Propagules

Hydrilla verticillata (L.f.) Royle is a monocotylednous plant belonging to the Hydrocharitaceae family (frogsbit). It is a submersed vascular macrophyte that is perennial in

Figure 1. Vegetative portion of hydrilla (*Hydrilla verticillata* [L.f.] Royle) showing shoots and roots extending from the stolon.

nature. Hydrilla grows rooted in the hydrosoil, sending out long branching stems. The lower leaves are opposite and small. The median and upper leaves are in whorls of four to eight. The leaves are verticillate, narrowly lanceolate, spreading, one nerved, and 1 to 2 cm long by 1.5 to 2 mm wide, serrate, and ending in a minute spine. The pistillate flowers of hydrilla are epigynous. Hydrilla has an entire style and three stamens. The flowers are never perfect. They arise from a spathe formed near the stem apex and are found at or near the water surface. The entire flower is inconspicuous, being 4 to 5 mm across. Seed formation, if at all, is poor. Reproduction is by vegetative fragments, tubers and turions. Excised shoots develop into new plants that attach to the hydrosoil by fine filiform adventious roots. Subterranean shoots have fleshly acute or acuminate scale-like leaves (4, 32,43,50).

Hydrilla propagules are located in axils of leaves (turions) and attached to subterranean stolons (tubers). The meristems of the tubers and turions are well protected. They are enclosed in many small scale-like leaves (40). These leaves can be separated from the meristem one by one without much difficulty. The apical meristem is housed at the apex of a single stem (Figure 2). This stem is attached firmly to the lower half of the tuber at approximately the point where the tuber begins to form a curve (Figure 3). The lower portion of the tuber is completely surrounded by a sheath of three to four leaves that are large when compared

Figure 2. Apical meristem of hydrilla (*Hydrilla verticillata* [L.f.] Royle) showing the vascular strands and scale-like leaves that surround the meristem (25X).

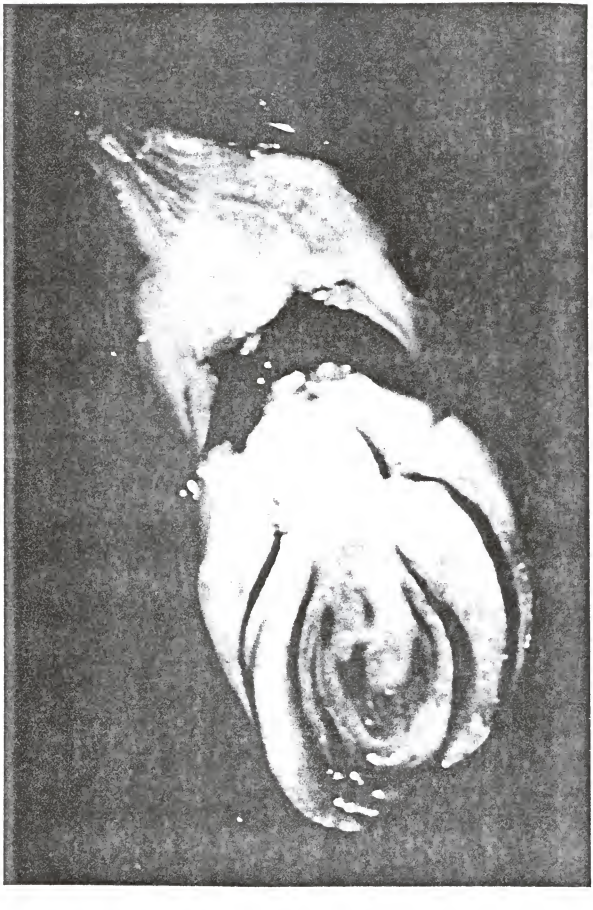
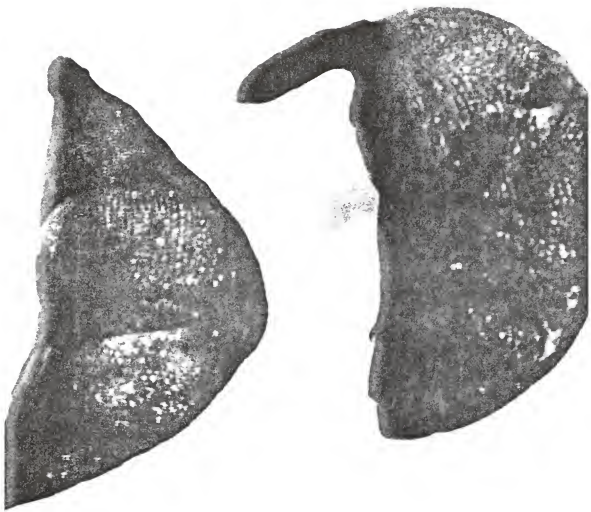


Figure 3. Tuber of hydrilla (*Hydrilla verticillata* [L.f.] Royle) (25X).



to the leaves that enclose the apical meristem. The greatest apparent difference between tubers and turions, other than location on the plant, is that the turion is green in color and somewhat smaller than the tuber (42).

Literature Review

Many studies have been conducted with tubers of terrestrial plants, but the number that have been devoted to hydrilla tubers is miniscule. The majority of the tuber studies have been done with plants such as the white potato (*Solanum tuberosum*) (1,8, 9,12), and nutsedge (*Cyperus rotundus*) (46,56,61).

Tubers are enlarged ends of underground stems or, less often, roots consisting mainly of parenchyma cells containing large amounts of stored polysaccharides. The tuber may occur as a swelling of a cluster of stem internodes. The nodes are retained as eyes with bract and subtended bud (31).

Tuber formation is first detectable when cells of the youngest expanding internode on the underground stolons begin to expand radially, rather than along the stolon axis. Most of the tuber volume comes from secondary cell division proceeding throughout all tissues. The initiation of tuber development is favored by short days, a shortage of nitrogen and phosphorus, high radiation, and low temperature (16,29, 31).

There are two main theories about the causes of tuberization in the potato and other species of plants: (1) tuberization is primarily controlled by the levels of substrate available for growth at the stolon tips; and (2) that it is

hormonally controlled (36,52). Experiments by Kumar and Wareing (29,30) show evidence for the existence and movement of a specific tuberization stimulus. They showed that the stimulus could move basipetally and acropetally, and that tuber development occurs only at the physically lowest nodes of stem cuttings, whether they are normally orientated or inverted. The development of basal axillary shoots of *Solanum andigena* as stolons is dependent upon their being subject to apical dominance. In 1975, Paterson (48) looked at the effects of a carbon dioxide-enriched internal atmosphere on tuberization. He found that exposure of the root system of potato plants to 80% carbon dioxide/20% oxygen atmosphere for 12 hours resulted in the formation of significantly more tubers than his controls. Treated plants showed increased tuberization along the length of stolons. High levels of carbon dioxide in the internal atmosphere of potato tubers were accompanied by physiologically active levels of ethylene.

A tuber is considered dormant when it is placed under suitable conditions for growth and growth does not occur. This inactivity could result from an excess of growth-inhibiting, over-stimulating substances within the tissues. In the natural environment, there are many factors that may lead to a loss of dormancy. These include: (1) prolonged exposure to low temperature; (2) periods of alternating temperature; (3) the leaching of water-soluble substances; (4) the gradual loss of the capacity to produce inhibitors; (5) light; and (6) water imbibition.

Dormancy is a significant part of the life cycle of tubers, corms, and aerial buds. It is necessary that the nondormant tuber take up water at a non-limiting temperature and that there is sufficient oxygen uptake. When full turgor occurs, protein, and cell wall materials swell, and metabolic activity is accelerated. Initially, rates of gas exchange are low but increase with the uptake of water.

The metabolism of tuber tissue appears to change from synthesis and deposition during growth and storage to a significant increase in hydrolytic reactions at the end of dormancy. When tubers continue to be maintained in storage, apical buds elongate at rates dependent upon temperature. The growth rate depends on the reserve substrate available in the tuber and the rate at which the substrate is hydrolyzed. This process is dependent upon some stimulus from the growing sprout. Externally supplied gibberellins will also initiate these changes. When there are many sprouts, the smallest sprout stops growing due to inhibition by apical dominance (38).

Kumar and Wareing (30) looked at factors controlling stolon development in the potato plant. Some conditions that favor stolon development are darkness, high humidity, and apical dominance. High gibberellin levels also promote stolon development, which is necessary to get tuber formation.

Wismewski (67) experimented with winter buds of aquatic plants. He found that the rest period of buds of *Hydrocharis morsusranae* could be shortened by wounding and lengthened by darkness. *Hydrocharis* formed buds the entire winter when

kept in darkness and immersed in rain water or a nutritive solution. Appleman (1) found that potato extract contained active diastase at all times during the rest period. He also found that the extract from new potatoes showed less ability to accelerate the oxidation of pyrogallol than extract from tubers at the end of the rest period.

Sachs [cited in (1)] reported that the rest period was caused by a deficiency of soluble food and the cessation of rest was due to a gradual production of enzymes which solubilized stored food reserves. Johannsen (26) found that ether and chloroform treatment for tubers was only effective for promoting growth at the beginning and near the end of rest periods. Molisch (44) got early opening of buds by immersing shoots in water at 35 C. Jesenko (25) found that hydrochloric, sulfuric, and tartaric acids, as well as alcohol and water, saturated with carbon dioxide hasten germination of buds of a number of woody plants. Jost (27), working with wounded potatoes, indicated that these potatoes showed earlier sprouting buds than unwounded ones.

Askenasy in 1877 (2) considered the yearly periodicity of plants a property that was due entirely to external conditions. Schimper in 1898 (51) looked at the behavior of tropical vegetation in respect to growth and rest period. In places where moisture and temperature conditions were favorable during the entire year, many plants showed a rhythmic alternation of periods of rest and growth processes. He considered internal causes as responsible for the alternation

of rest and activity in a nearly uniform climate. Kidd (28), working with oxygen, nitrogen, and carbon dioxide, found that oxygen in concentrations above 5-10% was harmful to potato. This harmful action of oxygen was enhanced by the presence of carbon dioxide. Carbon dioxide was found to inhibit sprouting of tubers at a concentration of 20%. Higher concentrations of carbon dioxide cause marked injury and death.

Thornton (60) found carbon dioxide increased oxygen uptake in both dormant and nondormant potatoes. When potatoes were removed from carbon dioxide treatment, healthy plants were produced that gave no indication of having been injured by the previous treatment. When tubers were stored in carbon dioxide, the carbon dioxide output by tissue was less than the oxygen uptake. Thornton's results also showed that carbon dioxide in the presence of oxygen does decrease the hydrogen ion concentration to a marked degree. The extent of pH change of tissue was influenced to a large degree by the length of storage period in carbon dioxide. Storage of tissue in air after treatment with carbon dioxide resulted in the restoration of pH to the normal level. Enhanced sprouting of dormant buds treated with 57% carbon dioxide for 6 days occurred after a period of 19 days (8,59).

Immature tubers have a thin, slightly suberized skin which is permeable to water and gases. As the tubers mature the skin becomes more suberized and more adherent to underlying tissue. Dry conditions favor suberization while moisture retards it. Further studies by Appleman (1) showed that ethyl

bromide gas was effective in forcing resting buds of potatoes into growth. The rest period could also be shortened by wrapping new tubers in cotton saturated with hydrogen peroxide.

Oxygen is present in both the atmosphere of intercellular spaces and dissolved in the cell sap. The solubility of oxygen in the cell sap averages 87% of its solubility in distilled water. There is little change in the oxygen content of tubers during storage. Oxygen is thought to play no role in the onset or, under ordinary storage conditions, termination of dormancy (9). Thornton (58), on the other hand, thought dormancy was terminated by restriction of the oxygen supply because of increased suberization of the periderm. He showed that sprouting could be induced by reducing the amount of oxygen in the storage atmosphere to between 2 and 10%, depending upon the humidity.

Palmer (47) found that oxygen levels below 20% reduced sprouting of nutgrass tubers, but at 20% oxygen obtained 80% sprouting. Tubers at 100% carbon dioxide or nitrogen failed to sprout. A 100% oxygen level induced 95% sprouting. The tubers remained viable under 1 and 10% oxygen. Palmer (46) found total organic acids high in dormant tubers but they declined after the first 2 days of germination follwed by a rapid increase for 6 to 10 days. Citric, malic, and oxalic acids remained constant, with citric content being greater than malic and the malic content being greater than oxalic. The fats declined until the 6th day and increased to the 8th day. Soluble carbohydrate content showed a gradual decline

after 2 days. The changes in substrates and enzyme activities indicated that dormant tubers metabolize predominantly organic acids. Dormant tubers had a respiratory quotient of 2.1 which increased to unity in 5.5 days after germination. Taylorson (56) reported that in yellow nutsedge tubers, the lowest level of reducing sugars occurred in August and the highest occurred in May.

Carbon dioxide stimulates tuberization of isolated potato stolons cultured *in vitro*. This stimulation is inhibited by ethylene (39). Ethylene causes diageotropic growth, terminal hook of the stolon, and inhibition of root elongation (47). Ethylene appears to have dual effects on potato dormancy and sprout growth in that it shortens the duration of the rest period and inhibits the elongation of sprouts during extended treatment. Ethylene in the form of ethylene chlorohydrin also causes an increase in sucrose and a decrease in reducing sugars in potatoes and gladiolus (11,13,37). Tubers that were cut and planted at once after treatment with ethylene chlorohydrin rotted, but those planted after subsequent storage in air sprouted (14).

Chemical substances other than gases cause a response in tuber dormancy and tuberization. Teo and Nishimoto (57) found that bud sprouting and growth of purple nutsedge are altered by benzyladenine. Benzyladenine increased the number of sprouts per tuber while ethepon (ethrel) (2-chloroethylphosphonic acid) had no stimulatory effects on sprouting at 10, 100, and 1000 mg/l. Esashi and Leopold (15) found stim-

ulation of tuber growth by pretreatment of tubers with cycloheximide before cytokinin application.

Stoller and Wax (55) observed that the minimum temperature for yellow nutsedge tuber germination in the laboratory was 12 C. About 50% of the tubers retained viability after cold treatment for 4 to 48 h at temperatures less than 0 C.

Gibberellins stimulate cell division and are natural plant components that may be bound or in an altered form in the plant. Gibberellins also induce flowering, fruit set, and suppression growth of lateral buds and root formation. There is no polar movement of gibberellins in the plant. Biran *et al.* (5), working with tuberization of dahlias, found that treatments with abscissic acid, succinic acid-2-2-dimethylhydrazide, or 2-chloroethylphosphonic acid promoted tuberization during long days and that gibberellic acid inhibited tuberization. The influence of these chemicals on tuberization during long days was not, however, as great as during short-day conditions.

Temperature and light are two important physical factors that affect tuber formation and sprouting. In many cases, the formation of tubers can be regulated by photoperiod, light, and temperature.

Van Schreven (63) presprouted potato tubers in the dark at a low growth temperature and stimulated premature tuber formation. The low temperature caused retardation of shoot and root formation. Premature tuber formation correlated with conditions which slowed root and sprout formation but not with

loss of reserve materials in the mother tuber. Tubers that had been kept at 2 C for several weeks contained a much higher level of reducing sugars and sucrose than tubers kept at room temperature. At low temperatures, 0 C to 10 C, saccharose and reducing sugars, glucose and levulose, accumulated in tubers due to hydrolysis of starch. Fresh tubers may contain up to 5.23% reducing sugars and 7.28% total sugars. When they are kept at 0 C the proportion of insoluble nitrogen is 8 to 12.5% of the total nitrogen. Crystalline products from potato tubers included tyrosine, asparagine, and glutamine.

Temperature affects the carbon dioxide content of plant tissues. At 20 to 37 C, the carbon dioxide content increases. Storage temperature seems to have an opposite effect on the oxygen content of potato tissue. An increase in temperature leads to increased respiration of tubers. The higher the temperature the lower the solubility of oxygen in the cell sap. The solubility of oxygen at 37 C is a little more than half the solubility at 5 C. An oxygen deficit in the intercellular spaces can be expected to double for every 10 C rise in temperature (9). Temperatures of 20, 25, and 30 C gave enhanced sprouting when tubers were dipped in ethylene chlorohydrin; however, at 35 C tuber seed pieces were killed (12).

CHAPTER II
GENERAL METHODS AND PROCEDURES

Introduction

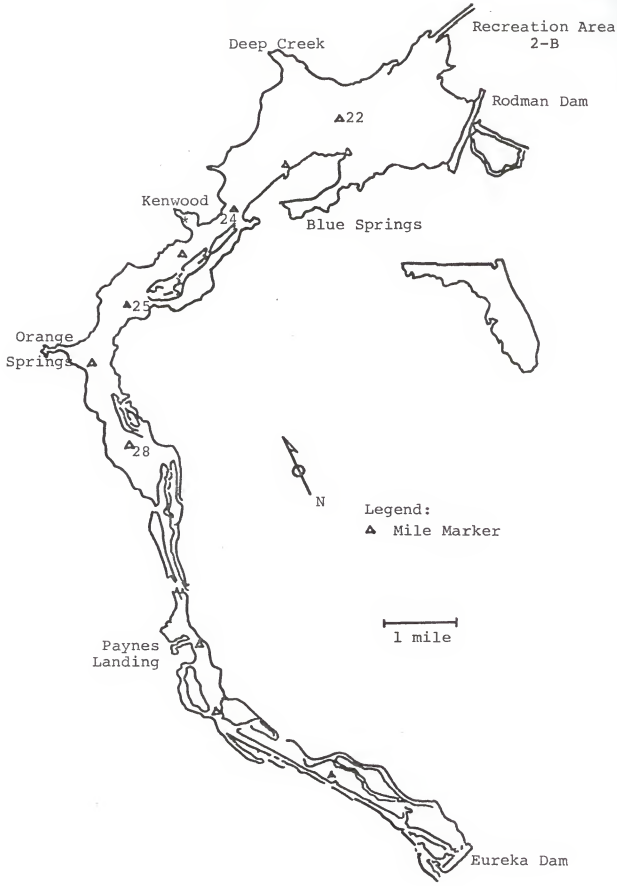
Tubers used in these studies were harvested from several locations. Some came from plastic tanks at the Fort Lauderdale Aquatic Research Center in Fort Lauderdale, Florida, others from plastic pools located on the University of Florida campus but the majority were harvested from the Rodman Reservoir located in north central Florida (Figure 4).

Harvesting Hydrilla Tubers

Hydrilla tubers were generally harvested in the shallows by using a shovel to trench along the hydrosoil. A plastic 10.0 cm diameter core sampler was used to obtain tubers from deeper locations. A 1.25-cm mesh screen was placed over a 189-liter drum that was open at both ends. A small pump equipped with hose provided the water needed to wash away the hydrosoil.

The tubers are usually sampled from a water column depth of approximately 60.0 cm and a hydrosoil depth of 10.0 cm. Once the tubers were harvested they were stored in moist peat until time for use in the laboratory. Immature or small tubers were separated from the mature ones.

Figure 4. Map of Rodman Reservoir (Lake Ocklawaha) at eighteen feet mean sea level. Asterisk indicates area where *Hydrilla verticillata* (L.f.) Royle tubers were collected (22).


Recreation Area
2-B
Deep Creek
Rodman Dam
22
Kenwood
24
Blue Springs
25
Orange
Springs
28
N
Legend:
Mile Marker
1 mile
Paynes
Landing
Eureka Dam

Determination of Mineral Composition of Tubers

To determine ion composition, tubers were dried in a forced draft oven at 70 C for 72 h, then ground in a Wily mill to pass a 60-mesh sieve. Samples of 10 g dry tissue were sent to the University of Florida Analytical Research Laboratory where they were analyzed in duplicate for Al, Ba, Ca, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Si, Ti, and Zn by atomic absorption spectroscopy (Perkin-Elmer, Model 303). A flame emission spectrophotometer was used for K and Na.

Sprouting Tubers under Several Temperature Regimes

Tubers were sprouted routinely in a germinator at temperatures of 28-30 C. The germinator was equipped for light and dark conditions, having four fluorescent lights on each side of the unit providing a total light intensity of 40 μ E/m^2/sec.

To determine the optimal sprouting temperature for hydrilla propagules, a temperature-gradient bar developed by West, Biggs, and Baskin (66) was used. The bar was composed of aluminum alloy with dimensions of 13 cm x 2.4 meters x 5 cm. The bar contained nine holes across at each temperature position. Each hole was large enough to accommodate one 10-ml beaker. Both ends of the bar were turned downward at a 90° angle. One end was placed in ethylene glycol that was maintained at -7 C by a refrigeration unit. The other end was in a water bath at 60 C, maintained by an electric heating element. Ten-ml beakers containing tubers covered with deionized water were placed directly on the bar. Temperature along the

bar was monitored by glass thermometers. For dark conditions, the bar was completely covered by a styrofoam top. For light conditions, a fluorescent bulb was placed 60 cm above the length of the bar. The tubers were left on the temperature-gradient bar for 14 days.

Sprouting Tubers under Different Atmospheric Compositions

Tubers that had been harvested from 60.0 cm depth of water column were placed in 250-ml Erlenmyer flasks, covered with deionized water, and flushed for 1 min with either carbon dioxide, nitrogen, or oxygen. Flow meters were used to control the quantity of nitrogen and carbon dioxide when they were used together. The Erlenmyer flasks were sealed by placing rubber stoppers in them and then covering the stoppers with plastic wrap which was held tightly by a rubber band. Tubers that were under aerobic conditions were left unsealed. The tubers were then placed in a germinator under continuous light or continuous dark at 30 C.

Determination of Respiration of Mature Hydrilla Tubers

Respiration for tubers was determined by use of the Warburg Constant Volume Respirometer (62). Three tubers were placed in each reaction flask along with 2 ml of deionized water. Two-tenths ml of KOH was placed in the center well. A small piece of carefully folded filter paper was also placed in the center well to increase the surface area for the KOH. Respiration was measured for 1 h at 25 C.

Drying Hydrilla Tubers

Freshly harvested mature tubers were washed in tap water, then soaked in 1% $NaHClO \cdot 5H_2O$ for 1 min. The tubers were then rinsed three times in deionized water and blotted dry. They were then weighed and placed in opened Petri dishes that were placed in sealed plastic bags containing anhydrous $CaSO_4$. After drying for specified time periods, the tubers were again weighed and covered with 10 ml deionized water. The Petri dishes were placed in a germinator at 30 C under continuous light for 14 days. Sprouting was recorded for each drying time period.

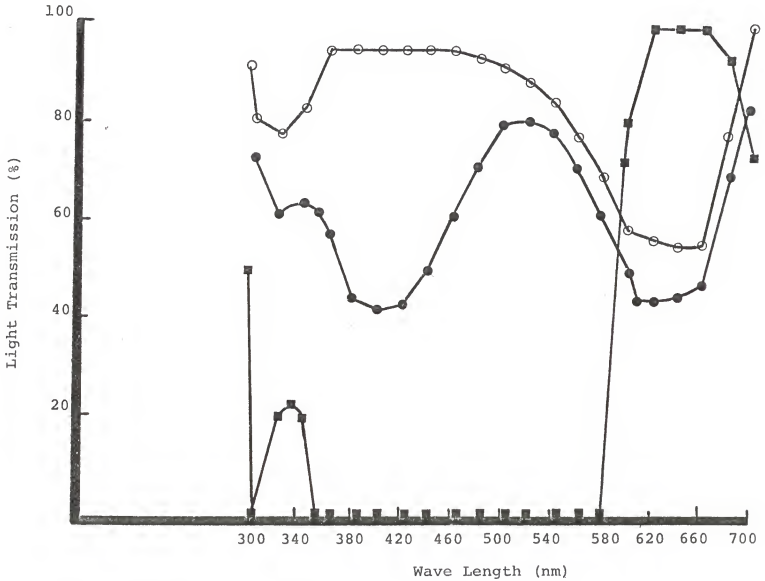
Determination of the Effect of Light on Hydrilla Tuber Sprouting

Four replicates of 15 mature tubers each were placed in Petri dishes and covered with deionized water. The Petri dishes were placed inside four open-topped boxes that were each covered with a different color filter paper. The filters were blue, red, green, and white. The square boxes were made of wood and were 30 cm wide and 45 cm in height. The light source was six 40-W fluorescent bulbs that were 75 cm above the tubers. Light intensity was determined by using a Lambda quantum meter, Model LI-185. The absorbance of the filters is indicated in Figure 5. The tubers were sprouted at a temperature of 25 C.

Nitrogen Determination

Total nitrogen was done by the salicyclic acid modification using a micro-Kjeldahl apparatus (24). Dried plant

Figure 5. The transmission of light through red, green, and blue filters. Open circle is blue filter, filled square is red filter, and filled circle is green filter.


100
80
60
40
20
Light Transmission (%)
300
340
380
420
460
500
540
580
620
660
700
Wave Length (nm)

material was ground in a Wily mill to pass a 20-mesh sieve. Two-tenth g samples were placed into seven digestion flasks along with 0.5 g of anhydrous $Na_2S_2O_3$, 1.1 g of catalyst (K_2SO_4+$CuSO_4$), and 5 ml of H_2SO_4- salicyclic acid. The mixture was swirled and placed on the digestion unit. The samples were digested at 100 C for 10 min, then at 85 C for an additional 60 min.

After digestion was completed, the samples were steam distilled. First, 25 ml of deionized water was added to each sample, which was then placed on the distillation stand. Twenty ml of 10 M NaOH was then added through the top funnel of the unit. Thirty ml were distilled into a separate 50-ml flask containing 5 ml of boric acid-indicator. The sample was then titrated with 0.114 N H_2SO_4.

Nitrate nitrogen (NO_3-N) in water taken from a pool where tubers were being actively formed was determined with a digital pH meter, Model 801, with a specific ion electrode. The NO_3-N standards were 14.0, 7.0, 1.4, and 0.14 mg/l NO_3^-. Chloride and ammonia were determined also by this same method. Phosphate (PO_4) was determined with a Beckman DB-G grating spectrophotometer at a wave length of 660 nm by the method of Truog (10). A Beckman Conductivity Bridge, Model PC 16B2, was used to determine the total salts.

Determination of Sucrose and Reducing Sugars

The Nelson-Somogyi copper reduction method was used to measure both reducing sugars and sucrose (45,53).

Eighty percent ethanol-soluble carbohydrate from 50 mg ground tuber tissue samples were used. Beakers containing the ethanol solution were placed on a hot plate and brought to a slight boil. Once all the alcohol was evaporated from the solution, the remainder was transferred to a 10-ml graduated cylinder. The purpose was to get all the sugars into a 10-ml aqueous remainder. The solution was then transferred to a 15-ml polypropylene centrifuge tube and was centrifuged at 27,750 X g for 1 h. After centrifugation, the supernatant was decanted into glass test tubes. Two sets of tubes were used. Included in each set was a blank (0.5 ml water for sucrose and 1.0 ml water for reducing sugars), a series of standards for glucose (0.5 ml glucose + 0.5 ml water/tube), and triplicate sample tubes for each unknown sample (0.5 ml aliquots/tube for sucrose, 1.0 ml aliquots/tube for reducing sugars). To each of the tubes used for sucrose determination was added 0.5 ml of invertase mix. The invertase mix was made from the following basic ratio: 0.5 ml invertase concentrate in glycerol plus 3.0 ml of water. Each of the sucrose tubes was allowed to incubate at room temperature for 90 min after which 1 ml of copper reagent was added. The samples were then boiled for 30 min. A 1.0-ml aliquot of dilute arsenomolybdate reagent was added to each tube. Water was added to make the total volume in the tube 5 ml. The absorbance was determined at 520 nm on a Klett-Summerson Photoelectric Colorimeter, Model 800-3.

Determination of Starch

Starch in hydrilla tubers was determined by the method of Hassid and Neufeld (20). Dry hydrilla tubers were placed in a ventilated oven with a fan to circulate air over tissue at 79-80 C. The dry material was ground to pass a 50 to 80-mesh sieve. A 50-mg sample was placed in a 50-ml centrifuge tube, a few drops of 80% ethanol were introduced to prevent clumping, then 5 ml of water were added. To extract the soluble sugars, the tube was immersed in a hot water bath. Twenty-five ml of hot 80% ethanol were added and the extract centrifuged. The ethanolic solution was decanted. This was repeated three times to completely remove soluble sugars, and the extracts were combined. The sugar-free residue was heated 15 min with 5 ml of water in a boiling water bath in order to gelatinize the starch. The suspension was cooled to room temperature, and 6.5 ml of 52% perchloric acid was added while stirring. The tube was kept cool by immersion in a water bath (25 C); the stirring was continued about 5 to 15 min. Twenty ml of water were then added, and the sample was centrifuged. The aqueous starch solution was decanted into a 50-ml volumetric flask. Extraction with perchloric acid was repeated without preliminary heating. The supernatant was decanted through glass wool then diluted to the mark and centrifuged. A 10-ml aliquot of starch extract was transferred to a 50-ml conical centrifuge tube; 5 ml of 20% NaCl and 2 ml of I-KI reagent were added to the solution and mixed. The solution was allowed to stand for 20 min, then centrifuged. The supernatant

was then removed with extreme care. The precipitated starch-iodine complex was suspended in 5 ml of ethanolic NaCl. The solution was washed and centrifuged. The supernatant was discarded. To the packed precipitate, 2 ml of 0.25 N ethanolic NaOH was added. The liberated starch was centrifuged and washed several times with 5-ml portions of ethanolic NaCl as before. Five ml of hot water was added to the precipitated starch in the tube; this was stirred with a glass rod until the starch had dissolved. The solution was then transferred to a 50-ml volumetric flask.

To determine the starch concentration, aliquots of starch solution expected to contain 20-200 μg of starch were pipetted into acid-washed test tubes and diluted with water to 2.0 ml. At the same time, a series of tubes containing 0-200 μg of D-glucose in 2 ml of solution were prepared. The tubes were immersed in cold water, and 10 ml of anthrone reagent were added to each from a buret. The reactants were well mixed in the tube. The tubes were then placed in a boiling water bath for 15 min until the maximum color developed. The tubes were rapidly cooled by immersion in cold water. Absorbance was measured at 620 nm using a Klett-Summerson Photoelectric Colorimeter, Model 800-3. The amount of starch was calculated from the standard curve of D-glucose, and was multiplied by 0.90.

Application of Herbicides and Growth Regulators

Mature tubers that were harvested during the month of May, 1975 were subjected to the liquid forms of banvel (3,6-

dichloro-o-anisic acid) (0.82 kg/3.8 liter active ingredient), eptam (S-ethyl dipropylthiocarbamate) (2.7 kg/3.8 liter active ingredient), endothall (the mono[N,N-dimethylalkylamine] salt of 7-oxabicyclo[2,2,1] heptane-2,3-dicarboxylic acid) (0.91 kg/3.8 liter active ingredient), and ethrel (2-chloroethylphosphonic acid) (0.24 g/ml). These herbicides were diluted to the desired concentrations with deionized water. Four replicates of 10 tubers each were made for each concentration of herbicide.

The growth regulators used were gibberellic acid, maleic hydrazide, napthaleneacetic acid, and thiourea. Four replicates of 10 tubers each were made for each concentration.

The tubers were sprouted in Petri dishes containing the specified herbicide or growth regulator. The Petri dishes were left in a germinator under continuous light at 30 C for 7 days.

CHAPTER III
CHARACTERISTICS OF HYDRILLA TUBERS HARVESTED AT RODMAN RESERVOIR

Introduction

Characterization of hydrilla tubers is important because they are numerous, able to remain dormant *in situ* for extended periods, and are the major source of reinfestation. Hydrilla tubers are present in the Rodman Reservoir during all seasons of the year. Once the tubers are collected and placed under controlled conditions in the laboratory some appear to be dormant for as long as 9 months, but eventually a high percentage of them will sprout.

Characterization is also important to appliers of herbicides because once dormancy is broken the tubers can reinfest an area within a week to 14 days.

Materials and Methods

Hydrilla tubers were harvested at the Rodman Reservoir as described previously. A core sampler 90 cm in length, 10 cm in diameter, was used to get estimates for the number of tubers/m^2. Only mature tubers were used to make estimates.

The mineral nutrient content of hydrilla tubers was determined by using atomic absorption spectroscopy. Water analysis was done by the method of Jackson (24). Starch, sucrose, and reducing sugars were determined as described

previously. Lipids were determined by the analytic method of Official Analytical Chemists (3). Nitrogen and crude protein were determined by the Kjeldahl method (24).

Results and Discussion

The Location of Tuber Formation

Tubers are formed approximately 8 to 10 cm below the hydrosoil, where they become detached from the parent plant and remain dormant for an indeterminate length of time. The tuber is considered the primary mode of reinfestation by this plant because of its dormancy and inaccessibility to various types of herbicides. Hydrilla tubers are probably found at any depth that the vegetative portion of the plant will grow. Tubers formed at 0.6 m in water column depth show more vigorous sprouting rates than tubers harvested from other depths.

Some Characteristics of Tubers

In characterizing tubers, the depth of water column, depth of sample in hydrosoil, average number of tubers/m^2, average fresh weight, average volume, and the average density of tubers were measured (Table 1). Twenty-eight independent core samples were collected, yielding 135 mature tubers. These tubers were taken from a water column depth of 60.0 cm and a hydrosoil depth of 30.0 cm. The average number of tubers collected amounted to 4.8 per core. This was approximately 580 tubers/m^2. The average fresh weight was 0.21081 g/tuber, the average volume 0.17481 cc, and the average density 1.20589 g/cc. Data of this type may vary with the seasons of the year

Table 1. Some characteristics of hydrilla tubers harvested at Rodman Reservoir during January.

Depth of Water Column	0.6 m
Depth of Sample in Hydrosoil	0.3 m
Average Number of Tubers	4.8/core
Average Number of Tubers	$580/m^2$
Average Fresh Weight of Tubers	0.21081 g/tuber
Average Volume of Tubers	0.17481 cc
Average Density of Tubers	1.20589 g/cc

Note: The above values were calculated from 28 independent core samples containing a total of 135 tubers.

and with location. Hydrilla seems to form tubers well until the month of April[1], after which there is vigorous growth of the vegetative portion of the plant, especially during the summer months. There appears to be an inverse relationship between vegetative growth and propagule production during the summer growth period. Hydrilla flowers in the Rodman Reservoir in October[2] and forms tubers until the beginning of April. While the lush growth is occurring during the summer months, there is an increase in the sprouting of tubers that were formed previously.[3] This phenomenum was observed after the water was raised in Rodman Reservoir. This was true especially in shallow areas.

Mineral Nutrient, Carbohydrate, Nitrogen, and Lipid Composition of Tubers

Hydrilla does extremely well under eutrophic conditions. The nutrients highest in hydrilla tubers are calcium, potassium, magnesium, and iron (Table 2). As expected, calcium was the highest nutrient found in tubers. The vegetative portion of the plant does well in calcareous locations. The chemical composition of hydrilla tubers is similar to that of other tubers, the highest component being starch followed by crude protein, lipids, sucrose, reducing sugars, and nitrogen (Table 3). A chemical analysis of waters where tubers were actively being formed (Table 4) indicated that

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[1,2]Personal communication, Dr. William T. Haller, 1975, Agronomy Department, University of Florida, Gainesville.
[3]Personal observation, Rodman Reservoir, 1974-75.

Table 2. Mineral content of hydrilla tubers.

Nutrient	mg/g dry wt.
Calcium	3.64
Potassium	3.38
Magnesium	0.73
Iron	0.61
Sodium	0.51
Silicon	0.05
Zinc	0.04
Copper	0.04
Aluminum	0.04
Manganese	0.01
Chromium	0.01
Lead	0.01
Cobalt	0.003
Titanium	0.00
Nickel	0.00

Table 3. Gross organic chemical composition of hydrilla tubers.

Component	Dry wt. (%)
Starch	46.80
Crude Protein	5.30
Lipids	1.00
Sucrose	4.17
Reducing Sugars	0.39
Nitrogen	0.86

Table 4. Analysis of water in which hydrilla tubers were actively being formed.

Component	μmohs/cm	mg/l	pH
Total Soluble Salts	77.22		
NO_3-N		0.32	
Hydrogen Concn.			7.2
Chloride		24.00	
Phosphate (PO_4)		0.60	
Potassium		2.66	
Ammonium Ion (NH_4)		0.25	

hydrilla is able to survive in waters low in nutrients. Mitra (41) also indicated that hydrilla plants can grow under a wide range of conditions.

The Sprouting of Tubers under Continuous Light or Darkness

When placed under continuous light, tubers usually develop chlorophyll and start photosynthesizing. This may or may not be accompanied by sprouting. When the tubers are sprouted in darkness, the tubers remain pale, and the shoot is etiolated. It does not matter if the tubers are sprouted under light or dark conditions, for a mature plant usually develops within 14 days. However, the number of tubers sprouting in the dark is generally lower than the number sprouting in the light. Light, therefore, appears to have a stimulatory effect on sprouting.

The Meristem of the Tuber

The single meristem of the tuber is well-protected. It is enclosed in 17 to 18 small, scale-like leaves. The apical meristem is located at the apex of a single stem which is attached firmly to the lower half of the tuber at approximately the point where the tuber begins to curve. The lower outer portion of the tuber is completely surrounded by a sheath of three to four leaves. All the leaves contain a high concentration of storage material. Parenchyma cells are clearly visible on the surfaces of the leaves and form many rows across the entire length and width of the leaf.

The Effect of Water Drawdown on Hydrilla Tuber Sprouting

The effect of drawdown at Rodman Reservoir on tuber sprouting varied with depth of water column. Tubers that were harvested at 0.3 to 0.6 m depths before drawdown had 65% sprouting (18). After 2.5 months of drawdown, sprouting increased to 100%. What causes this change in sprouting appears to be carbon dioxide levels in the water and in the hydrosoil. A low carbon dioxide concentration in the hydrosoil apparently leads to increased sprouting of tubers, and a constant level of carbon dioxide keeps tubers dormant for long periods. In shallow sandy areas tubers sprout readily, whereas in areas where there is a lot of organic matter, many tubers remain dormant. This is evidenced by the number of tubers found in these different locations. In an area where black plastic was staked in the Rodman Reservoir, there was no tuber sprouting. When sampled with a core sampler, tubers at the rate of 500-800/m^2 were consistently harvested, and occasionally up to 2800 tubers/m^2 were harvested (18).

Drawdown tends to expose tubers at the shallower depths to light and less carbon dioxide. In many instances the tubers are completely dewatered. The area is usually moist enough for sprouting to occur. Drawdown promotes sprouting of tubers if it is done at the right time of the year.

Variability in the Quantity of Tubers from One Small Area to Another

There is extreme variability in the number of tubers found within a small area, although there are millions of

tubers/ha. A special technique of digging has to be applied in order to find tubers in the field because the stolons appear to run in bunches. In order to find tubers at depths of 60 to 90 cm, the best method is to dig a trench about 15 cm in depth for a short distance then start another beside it. A washing screen and water hose are invaluable in harvesting tubers. Three persons can usually pick up 1,000 to 2,000 tubers within a 2-h period.

CHAPTER IV
EFFECT OF WATER DEPTH ON HYDRILLA TUBERS COLLECTED FROM RODMAN RESERVOIR DURING DRAWDOWN

Introduction

Of the submersed macrophytes now present in Florida, hydrilla is perhaps the most difficult to control. This plant can be expected to be a nuisance for some time to come because of its competitive ability and capacity for vegetative reproduction. Haller and Sutton (19) reported that competition from other plants in the aquatic community is almost impossible due to the canopy produced by hydrilla and the presence of millions of propagules/ha. The tubers are considered the primary mode of reinfestation by hydrilla. Mitra (42) reported that once the tubers begin to sprout, it only takes 12 to 14 days for formation of a fully developed plant.

Tuberization of hydrilla occurs at various depths of the water column. The purpose of this study is to determine if there are sprouting differences among tubers formed at five different depths in the Rodman Reservoir during drawdown.

Materials and Methods

Hydrilla tubers were collected during January, 1975 from the Rodman Reservoir during drawdown. Mature tubers were taken from the water column at depths of 0.3, 0.6, 0.9, 1.2, and 1.5 m. The tubers were located 8 to 10 cm below the

hydrosoil. When possible, a square meter of the hydrosoil was excavated to a depth of 0.15 m; otherwise, samples were taken with a core sampler.

The excavate was washed on a screen to separate the tubers from the hydrosoil quickly. The tubers were then stored in plastic bags containing moist peat in the dark at approximately 24 C until used.

Fifteen tubers were placed in Petri dishes and allowed to sprout under either continuous light or darkness at 30 C for 14 days. For each depth and light or dark condition, four replicates were made. The light intensity under which the depth experiment was carried out was 12 $\mu E/m^2/sec$ (400-700 nm).

An experiment was conducted in which hydrilla tubers were sprouted under various light-quality regimes. This experiment was carried out as described previously. Four replicates of fifteen tubers each were made for each light-quality regime.

The third experiment concerned the effect of aerobic and anaerobic conditions on sprouting. Hydrilla tubers were sprouted aerobically and anaerobically under continuous light or darkness in 250-ml Erlenmyer flasks at 30 C. Two sheets of filter paper and 10 ml of water were placed in each flask containing fifteen tubers. Four replicates were made for each combination. The flasks were then flushed with nitrogen for 1 min and sealed with stoppers that were coated with lanolin. The stoppers were then covered with plastic wrap

secured with rubber bands. For aerobic sprouting, the flasks were left open.

Results and Discussion

Effect of Water Column Depth on the Size, Distribution, and Sprouting of Tubers

Tubers harvested from Rodman Reservoir at different depths during drawdown exhibited differences in sprouting, size, and number (Table 5). The number of tubers taken from 0.3 to 0.9 m varied from 293 to 605/m^2. The value at 0.9 m was computed. The average weight of hydrilla tubers increased from 188 mg at 0.3 m to 290 mg at 1.5 m in water column depth. The weight of tubers taken from a depth of 0.3 m was significantly different from the weight of tubers taken at the other four depths. The weight of tubers taken from depths of 0.6 and 0.9 m was significantly different from the weights of tubers taken from 1.2 and 1.5 m. A positive correlation ($r=0.866$, $P=0.005$) between weight and depth for tubers sprouted in the light existed. The correlation ($r=0.646$) of weight to depth for tubers sprouted in the dark was lower and not significant.

Sprouting of Hydrilla Tubers under Continuous Light and Dark Conditions

The sprouting of hydrilla tubers under light conditions was much greater than sprouting in the dark (Table 5). The best sprouting percentages obtained in either light or darkness were obtained with tubers taken from reservoir water column depths that ranged from 0.6 to 1.2 m. Tubers that were

Table 5. Light and dark sprouting, fresh weight, and distribution of hydrilla tubers taken from various depths.

Depth (m)	Tubers/m^2	Weight of tuber (mg)†	Sprouting (%) Light	Dark
0.3	293	188a	43a	10a
0.6	326	230b	87b	62b
0.9	605‡	236b	63ab	47b
1.2		273c	68ab	25a
1.5		290c	45a	13a

Note: Sprouting was conducted at 28 C either in the light ($12 \mu E/m^2/sec$) or in darkness. Sprouting was observed during a period of 14 days immediately after tuber harvest.

†Average tuber weight was determined from the weights of 120 randomly selected individual tubers. Values with different letters are significantly different from each other according to the Duncan multiple range test, p=0.05.

‡Calculated from core sample data.

taken from depths of 0.3 and 1.5 m and sprouted under continuous light were similar to tubers taken from depths of 0.9 to 1.2 m. The best sprouting rates occurred in tubers collected from 0.6 m. This trend was also observed for dark-sprouting of hydrilla tubers.

Quantity of Hydrilla Tubers/m^2

The number of tubers/m^2 increased with depth of water column up to 0.9 m. The reason for this may be the concentration of carbon dioxide in the water and hydrosoil. Carbon dioxide inhibits the sprouting of hydrilla tubers when the tubers are subjected to constant levels of carbon dioxide. The tubers formed at the deeper depths may remain dormant for longer periods of time due to the higher carbon dioxide content found at deeper depths. Low temperature could also inhibit the sprouting of tubers.

Effects of Light Quality on Hydrilla Tuber Sprouting

Light enhanced tuber sprouting; however, the color of light seems not to affect tuber sprouting at all (Table 6). Hydrilla tubers needed very little light to stimulate sprouting. Tubers may sprout very well in the dark, depending on the depth of the water column from which they are taken. The vegetative vigor of tubers sprouted in the light was much better than those sprouted in the dark. Tubers sprouted in the light developed chlorophyll whereas tubers sprouted in the dark retained their original color. Blackburn *et al.* (7) reported that light is the most important physical condi-

Table 6. Effect of different light regimes on hydrilla tuber sprouting.

Light Regime	Light Intensity ($\mu E/m^2/sec$)	Sprouting† (%)
Blue	30	98a
Red	17	100a
Green	10	100a
White	30	100a

†Values are the average of four replicates. Values having the same letter are not significantly different according to Duncan's multiple range test, p=0.05. Tubers were sprouted at 30 C.

tion for aquatic weed growth. Submersed plants grow well at light intensities ranging from 1 to 4% full sunlight. Total radiation decreases with depth, and there are changes in light quality. Red light penetrates to greater depths in waters high in turbidity. If the conditions are favorable in the water, it takes very low light intensity to stimulate sprouting. Sprouting of hydrilla tubers is good under blue, red, green, and white light. The effect of light on tuber sprouting is apparent even under anaerobic conditions (Table 7).

Light stimulated the sprouting of hydrilla tubers under aerobic and anaerobic conditions. The sprouting percentages under light plus nitrogen and light plus air were similar. The sprouting percentages under dark plus nitrogen and dark plus air were also similar but significantly different from tubers sprouted under light conditions (Table 7).

Table 7. Light stimulation of hydrilla tuber sprouting under aerobic or anaerobic conditions.

Regime	Sprouting† (%)
Light plus:	
Nitrogen	97a
Air	93a
Dark plus:	
Nitrogen	58b
Air	63b

Note: Sprouting was conducted in 250-ml Erlenmyer flasks in a germinator at 30 C. Flasks containing N_2 were purged with the gas initially and stoppered while other flasks were left unstoppered for aeration. Each flask contained 15 tubers, and sprouting was recorded on the 14th day.

†Values are the average of 60 tubers. Values having different letters are significantly different according to Duncan's multiple range test, p=0.05.

CHAPTER V
EFFECTS OF TEMPERATURE ON HYDRILLA TUBER SPROUTING

Introduction

What are the conditions under which tubers are able to sprout? Do hydrilla tubers undergo a period of dormancy the same as other tubers? Steward (54) reported that once the tuber sprouted, it was able to develop for 85 days on stored food reserves alone. Hydrilla has been found at depths of 20 m (33) where the temperature of the water is expected to be constant with low light penetration. Mitra (41) reported that hydrilla grew at temperatures that ranged from 19.0 to 37.4 C. These are some of the conditions under which tubers have been surviving very well. The purpose of this experiment is to study the effects of temperature on hydrilla tuber sprouting.

Materials and Methods

Initially, hydrilla tubers for the first temperature experiment were obtained from Dr. David L. Sutton at the Aquatics Laboratory in Fort Lauderdale, Florida. Tuberization occurred in plastic pools and in man-made ponds located at the aquatics laboratory. The tubers were mailed to Gainesville, Florida and were used immediately since no means of satisfactory storage was then available. The tubers would develop a very lush fungal growth and decay if left at room

temperature or if placed at low temperatures. Tubers collected from Rodman Reservoir at a later time followed the same pattern.

The tubers were harvested from Rodman Reservoir at a water column depth of 0.6 m. Before use the tubers were soaked for 1 min in 1% $NaClO \cdot 5H_2O$, then rinsed several times in deionized water. The tubers were then placed in covered Petri dishes that contained two sheets of moist filter paper. The Petri dishes were placed in germinators set for constant temperatures of 10, 20, 25, and 30 C. Four replicates were made for each temperature. Each replicate contained 10 tubers. A total of 160 tubers was used. Tubers that were frozen for 2.5 months were kept at a constant temperature of 0 C. The tubers were sprouted under continuous light conditions.

In a second experiment, hydrilla tubers were sprouted on a temperature-gradient bar. The bar was made of aluminum, having a heating unit at one end and a refrigeration unit at the other. Nine holes were drilled in the bar for each temperature, and each hole was large enough to hold a 10-ml beaker.

Mature tubers were soaked in 1% $NaClO \cdot 5H_2O$ for 1 min, then rinsed several times in deionized water, after which five tubers were placed in each 10-ml beaker. Nine replicates were used at each temperature. The temperatures used were 9, 11, 14, 21, 25, 28, 31, 34, 38, 42, and 45 C. The tubers were left on the gradient bar for a period of 14 days. Two separate experiments were run, the first under conditions of continuous light and the second under continuous darkness.

Results and Discussion

Effects of Temperature on Hydrilla Tuber Sprouting

Temperature had a definite effect on tuber sprouting percentages (Table 8). Attempts to sprout tubers were initially done in the dark at temperatures of 10, 20, 25, and 30 C. The sprouting rates were 0, 55, 58, and 83%, respectively. When the tubers at 10 C were placed at 30 C in the dark, 60% of the tubers sprouted. Tubers that had been frozen for 2.5 months showed no sign of sprouting after a period of 14 days at any temperature.

Fully hydrated tubers did not sprout unless they were covered with water. This observation suggested that hydrilla tubers may require a state of anoxia for sprouting, which was achieved by covering the tubers with water. Also, it was observed that the shoot of tubers sprouted in the dark etiolated rapidly and the tubers retained their brownish color.

Optimal Temperature for Hydrilla Tuber Sprouting

Further experiments were conducted to find the optimal sprouting temperature for hydrilla tubers. This was accomplished by using a temperature-gradient bar, already described. Tubers that were placed in the light (Figure 6) at temperatures of 9, 11, and 14 C failed to sprout after a period of 14 days; however, when these same tubers were placed subsequently in a germinator at 30 C in the light, the sprouting was 100, 94, and 96%, respectively. At temperatures of 21, and 25 C, there was a gradual increase in sprouting, reaching a peak at 28 C.

Figure 6. *Hydrilla verticillata* (L.f.) Royle tubers that were sprouted on a temperature-gradient bar under continuous light conditions. The dotted line indicates tubers which had low temperature inhibition of sprouting reversed when the tubers were placed in a germinator under continuous light at a constant temperature of 30 C.

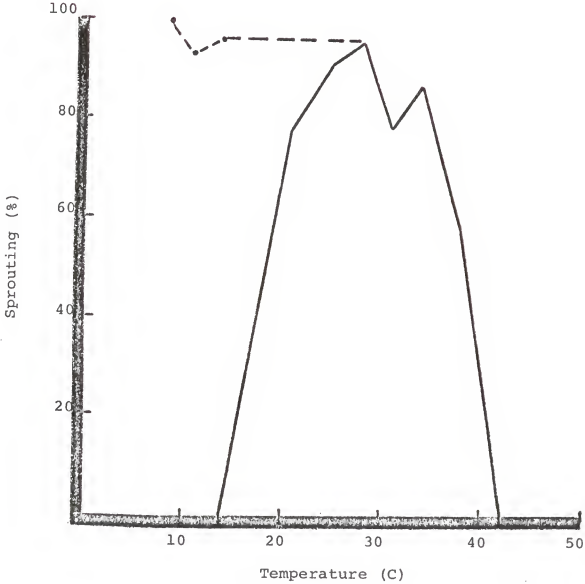

100
80
60
40
20
Sprouting (%)
10
20
30
40
50
Temperature (C)

Table 8. Effects of temperature on hyrilla tuber sprouting.

Temperature (C)	Sprouting (%)
10	0
20	55
25	58
30	83

Note: Values are the result of four replicates of 10 tubers each.

Above 28 C the sprouting percentage started to drop gradually. At temperatures of 43 C and 45 C, none of the tubers sprouted.

The sprouting percentages of hydrilla tubers placed in the dark (Figure 7) were similar to those found in the light. At temperatures of 10, 12, and 15 C, sprouting ranged from 2 to 10% after a period of 14 days. These same tubers were placed in the light in a germinator at 30 C. The sprouting after one week was 98, 88, and 98%, respectively. From 18 to 32 C, the sprouting was 100%. At 35 to 40 C, the sprouting percent was zero.

Low Temperature Inhibition of Hydrilla Tuber Sprouting

Apparently there is a low temperature inhibition of hydrilla tuber sprouting at temperatures of 10, 12, and 15 C. This low temperature effect is reversible when the tubers are placed at 30 C (Table 9). When tubers are exposed to a higher temperature (35 to 45 C), the effect is irreversible; the tubers completely deteriorate. The high temperatures bring about decay and breakdown of components of the tuber. .

Temperature effects on tubers seem to be important in the field. We can expect no germination when the temperature is too low. As the temperature of the water column approaches the optimum temperature for sprouting, more and more tubers will sprout, leading to reinfestation. The effect of temperature on sprouting is speculated to be on enzyme activity within the tuber, which decreases with low temperatures and increases with an increase in temperature. Nearly complete sprouting occurred in darkness from 18 to 32 C. In light optimal sprouting was at 28 C (Figure 7).

Figure 7. *Hydrilla verticillata* (L.f.) Royle tubers that were sprouted on a temperature-gradient bar under continuous dark conditions. The dotted line indicates tubers which had low temperature inhibition of sprouting reversed when the tubers were placed in a germinator under continuous light at a constant temperature of 30 C.

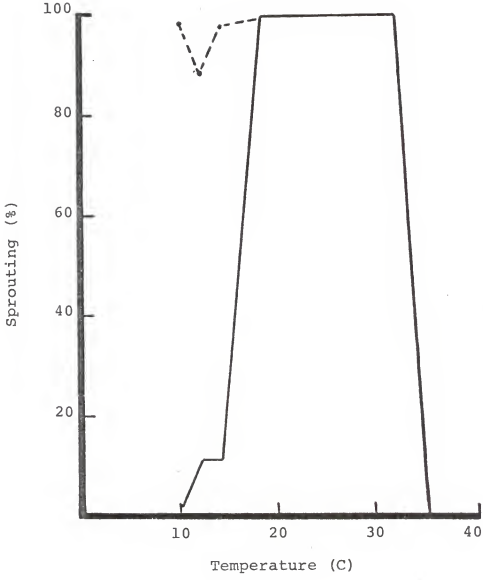

100
80
60
40
20
Sprouting (%)
10
20
30
40
Temperature (C)

Table 9. Reversal of low-temperature inhibition of sprouting of hydrilla tubers.

Initial Temperature††	Sprouting† (%) 7 days on bar		14 days on bar		5 days at 30 C†††	
	Tuber	Turion	Tuber	Turion	Tuber	Turion
10	2	0	2	0	98	95
12	0	0	11	0	89	95
14	2	2	11	2	98	84

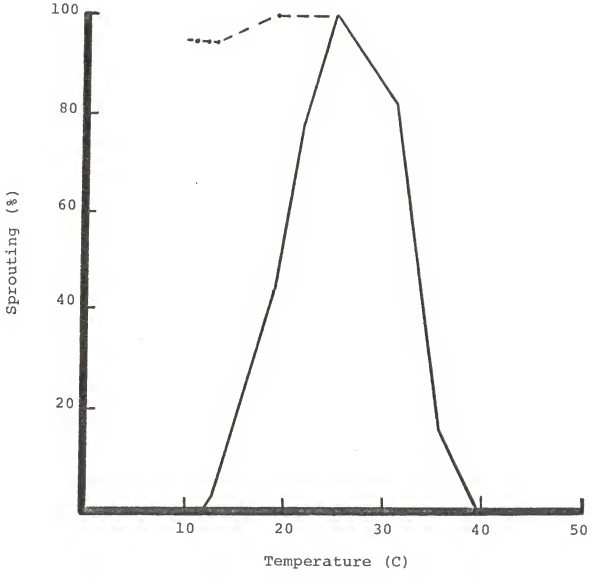
†Sprouting (%) based on 45-tuber sample size.

††Temperature on temperature-gradient bar during 14 days of observation.

†††Tubers and turions were placed in Petri dishes in the light in a germinator at 30 C.

Hydrilla tubers that were sprouted in the dark had an optimal sprouting temperature similar to tubers that were sprouted in the light (Figures 6 and 8). The turions also followed a similar pattern for reversal of low temperature inhibition.

Figure 8. *Hydrilla verticillata* (L.f.) Royle turions that were sprouted on a temperature-gradient bar under continuous dark conditions. The dotted line indicates turions which had low temperature inhibition of sprouting reversed when the turions were placed in a germinator under continuous light at a constant temperature of 30 C.


100
80
60
40
20
Sprouting (%)
10
20
30
40
50
Temperature (C)

CHAPTER VI
EFFECTS OF DIFFERENT STORAGE REGIMES ON HYDRILLA TUBER SPROUTING

Introduction

Tubers remain dormant in the hydrosoil for various periods and seem to be seasonal in regard to dormancy and sprouting. The objective of these experiments was to investigate some of the atmospheric conditions that promote dormancy and sprouting in hydrilla tubers and to look at some of the conditions that may cause the tubers to remain dormant in the hydrosoil for long periods.

Materials and Methods

Tubers used in this study were collected from Rodman Reservoir during the months of January, February, March, April, and August, 1975. Four replicates of 15 tubers were used. The tubers were collected from a water column depth of 0.6 m and a hydrosoil depth of 0.1 m.

When gases were used, the tubers were first covered with water and the vessels flushed for 1 min with the specified gas and then sealed. The tubers were sprouted in a germinator for 14 days at 30 C. The tubers were either under continuous light or continuous darkness.

When hydrilla tubers were stored in the dark under nitrogen, seven replicates and three controls were used. Fifteen

tubers were used for each replicate. This experiment was carried out in 250-ml Erlenmyer flasks that contained peat plus 10 ml of deionized water. The flasks were held in continuous darkness at ambient temperature (23 to 25 C). The tubers were left under these conditions for a period of one month.

Hydrilla tubers were stored in moist peat by placing the peat in plastic bags or in tin containers then placing the tubers in the containers. The containers were then shaken until the tubers were well dispersed.

Results and Discussion

Storage of Tubers under 100% Nitrogen

Inasmuch as light had a stimulatory effect on tuber sprouting under aerobic conditions, this first experiment was designed to find out what happens to sprouting under anaerobic conditions. Tubers collected during the month of January, 1975 were placed in sealed Erlenmyer flasks under conditions of continuous light plus nitrogen, continuous darkness plus nitrogen, continuous light plus air (unsealed), or continuous darkness plus air (unsealed). The tubers under conditions of light plus nitrogen exhibited 96% sprouting, which was similar to the sprouting percentage of tubers under conditions of light plus air (93). This same pattern followed for tubers sprouted in the dark. Under conditions of darkness plus nitrogen, the sprouting was 58%, and the darkness plus air was 63%.

In another experiment, tubers collected during the month of February were stored in the dark under nitrogen for 28 days. Under these conditions 68% of the tubers stored under nitrogen sprouted. None of the tubers in the controls germinated. The controls were sealed and contained only moist peat. This indicated some factor was present to keep the tubers dormant under anaerobic conditions. Nitrogen treatment had 65% sprouting.

Storage of Hydrilla Tubers under Nitrogen, Oxygen, and Carbon Dioxide

Experiments were run with tubers harvested during April. This time the tubers were placed under atmospheres of nitrogen in light or in darkness, carbon dioxide in light or in darkness, and oxygen in light or in darkness. Some tubers were placed in sealed and unsealed flasks in light or in darkness (Table 10). After a sprouting period of 14 days, all tubers under conditions of nitrogen plus light or nitrogen plus darkness had sprouting of 100%. Those under carbon dioxide plus light or dark showed no sprouting at all. Tubers that were in unsealed flasks under light or dark conditions showed sprouting of 97, and 70%, respectively. Tubers in closed flasks under light or dark conditions had a sprouting of 97%, and those under oxygen plus light or dark had sprouting of 85, and 12%, respectively.

Tubers stored in nitrogen, oxygen, or carbon dioxide and those that were sealed and stored in the dark showed no greening effect. Tubers that were under unsealed-light

Table 10. Effect of light and atmospheric gases on sprouting of hydrilla tubers and turions.

Sprouting conditions†	Sprouting on 14th day‡ (%)	
	Tubers	Turions
Light plus:		
Nitrogen	100 d	97 e
Carbon Dioxide	0 a	0 a
Oxygen	85 c	87 cd
Sealed Flask	97 d	100 e
Open Flask	97 d	100 e
Dark plus:		
Nitrogen	100 d	77 bcd
Carbon Dioxide	0 a	0 a
Oxygen	12 a	62 b
Sealed Flask (Air)	97 d	80 bc
Open Flask (Air)	70 c	95 de

†Germination at 30 C in 250-ml Erlenmyer flasks containing 10 ml of distilled water. Flasks were purged with 100% nitrogen, carbon dioxide, or oxygen, and were sealed with rubber stoppers and stopcock grease. Sealed and open flasks contained air and were sealed or left open, respectively. Light or dark conditions were continuous.

‡Values in the vertical column with different letters are significantly different from each other according to the Duncan multiple range test, p=0.05.

conditions turned green. Tuber decay was rapid under sealed-light conditions. The nitrogen-light treatment tubers decayed, but not as rapidly as those of the closed-light treatment. The tubers that were under conditions of oxygen plus light appeared firm and healthy, about the same condition as those of the open-light treatment. Tubers left under carbon dioxide for 14 days showed no response when placed in 500 mg/l GA_3 or in ethrel (100 mg/l). Tubers that were taken out of a carbon dioxide atmosphere and put under aerobic conditions also showed no sprouting response.

Storage of Hydrilla Tubers in Several Concentrations of Carbon Dioxide

Hydrilla tubers that were harvested during the month of August, 1975 were stored for 12, 24, 36, 58, or 60 h in carbon dioxide-nitrogen atmosphere. The concentrations of carbon dioxide were 0, 20, 40, 60, 80, and 100%, with concentrations of nitrogen to complete 100% of the volume. The storage times in the various levels of carbon dioxide and nitrogen generally had no effect on tuber sprouting when tubers were placed subsequently in Petri dishes for a week under continuous light at 30 C. The exceptions were tubers placed under 80 or 100% CO_2 for 36 h, 48 h, or 70 h (Table 11). Tubers that were left in sealed flasks continuously with carbon dioxide concentrations below 20% sprouted readily, exhibiting 90 to 95% sprouting. At carbon dioxide concentrations of 40%, sprouting was 5%, or at carbon dioxide concentrations of 60, 80, or 100%, none of the tubers sprouted. It appeared that under

Table 11. The effect of different storage periods in carbon dioxide and nitrogen on hydrilla tuber sprouting.

CO_2	N_2	Storage time in CO_2 and N_2 (h)					Control	
(%)	(%)	12	24	36	48	60	Sealed	Petri dishes
		------% Sprouting-----						
0	100	95	95	95	85	90	95	
20	80	95	100	90	100	90	90	
40	60	100	100	90	90	95	5	
60	40	100	100	95	100	95	0	
80	20	90	100	95	95	85	0	
100	0	100	90	80	80	30	0	
Air								95

Note: Twenty tubers were used at each CO_2 and N_2 concentration.

conditions of greater than 20% carbon dioxide, dormancy was induced in hydrilla tubers. Many of the tubers when taken out of the sealed flasks after a 5-week period, were still firm but showed no signs of sprouting after being placed in a germinator for a week at 30 C.

When hydrilla turions were subjected to the same regimes of light plus nitrogen, carbon dioxide, or oxygen or dark plus nitrogen, carbon dioxide, or oxygen, the turions responded in much the same manner as tubers, except under oxygen-dark conditions the sprouting percent of turions was greater than that for tubers (Table 10). When the turions that were under carbon dioxide were placed in Petri dishes under aerobic conditions, all the turions sprouted.

Storage of Hydrilla Tubers in Moist Peat for 4, 5, 7, and 8 Weeks

Tubers that were harvested from Rodman Reservoir during the months of January and February were stored until March, 1975. Before storage, the tubers were washed at the site of collection with reservoir water and placed in plastic bags containing moist peat. The plastic bags were sealed and stored in the laboratory at ambient temperature in the dark. While in storage the tubers remained firm; the only physical change that occurred appeared to be a change in the color of the epidermal layers of the tubers. At harvest time the peels were a creamy white color. After storage the epidermal layers were more of a brownish color.

Table 12. The effects of time of harvest on the sprouting of hydrilla tubers.

Date stored	Date taken out of storage	Storage time (weeks)	Sprouting (%)				
			Stunted	51 days	Consol-idation	4th day	14th day
Jan. 23	Mar. 24	8	72	18	99		
Feb. 4	Mar. 24	7	58	73	--		
Feb. 14	Mar. 24	5	52	15	55		
Feb. 26	Mar. 24	4	85	42	27		
Jan. 23	Oct. 13	35				100	
Feb. 14	Oct. 13	32				28	75
Feb. 26	Oct. 13	30				100	
Jun. 6	Oct. 13	15				43	95
Aug. 30	Oct. 13	6				58	65

On March 24, 1975, four replicates consisting of 15 tubers for each harvest date were put in Petri dishes and covered with water, and the tubers were then placed in a germinator at 30 C under continuous light conditions. By March 26, the tubers had turned green and elongated to 0.5 cm. The tubers remained in this condition of dormancy until May 14, approximately 51 days later. Of tubers that were harvested in January, 72% had stunted shoots; February 4, 14, and 26 had 58, 52, and 85% stunted shoots, respectively (Table 12).

After being in a germinator for 51 days, 18% of the tubers collected in January actually sprouted; 73% of those collected February 4; 15% for February 14; and 42% for February 26 sprouted. Tubers that had not sprouted were consolidated by harvest date, placed in 100-ml beakers and covered with deionized water. The samples were then placed back in the germinator under continuous light conditions at 30 C. After 7 days the tubers sprouted. Tubers harvested in January had a sprouting percentage of 99; February 14, 55%; and February 26, 27%. Tubers harvested on February 4 were not sufficient in number to make an estimate of sprouting percentage.

Storage of Hydrilla Tubers in Moist Peat for 6, 15, 30, 32, or 35 Weeks

These tubers were treated the same as those mentioned above. The tubers were harvested during January, February, June, and August, 1975 (Table 12). Four replicates of 10 tubers each were made for each harvest date. The tubers were put in Petri dishes, covered with deionized water and placed

in a germinator at 30 C. After 14 days the tubers that had been in storage 6, 15, 30, 32, and 35 weeks had sprouting of 65, 95, 100, 75, and 100%, respectively.

The tubers collected on February 14, 1975 seemed to be in perfect condition. The skins did not change color, nor did any of the tubers sprout while in storage; on the other hand, tubers harvested January 23, February 26, June 6, and August 30, 1975 exhibited sprouting while in storage of 70, 88, 16, and 27%, respectively (Table 13). Tubers collected February 14, June 6, and August 30 had slow sprouting, but after 14 days the sprouting increased.

Table 13. The effect of storage in peat on hydrilla tuber sprouting.

Date stored	Date taken out of storage	Tubers sprouted while in storage (%)	Number of tubers		
			Sprouted	Non-sprouted	Total
Jan. 23	Oct. 13	70	57	25	82
Feb. 14	Oct. 13	0	0	400	400
Feb. 26	Oct. 13	88	304	40	344
Jun. 6	Oct. 13	16	36	194	230
Aug. 30	Oct. 13	27	121	452	573

CHAPTER VII
EFFECTS OF DRYING ON HYDRILLA TUBER SPROUTING

Introduction

The drawdown of certain bodies of water is feasible and has been found in some cases to aid in the control of submersed macrophytes such as hydrilla (22,35,49).

The objective of this experiment was to determine the effect of drying on the sprouting of hydrilla tubers when they were placed subsequently under optimal conditions for rehydration and sprouting.

Materials and Methods

Mature tubers were collected from Rodman Reservoir at a water column depth of 0.6 m. The tubers were transported from the reservoir to the laboratory in plastic bags containing moist peat.

Hydrilla tubers were dried for various time periods in plastic bags that contained anhydrous $CaSO_4$ in flat glass trays. Ten tubers per replicate were used. There were five replicates for each drying period. Before the tubers were dried, they were soaked for 1 min in 1% $NaClO \cdot 5H_2O$, after which they were rinsed several times in deionized water. After the tubers were rinsed, the excess water was shaken off, and the tubers were patted dry with a paper towel. The tubers were then weighed and placed under drying conditions in open

Petri dishes. After the drying period was completed, the tubers were again weighed and placed in a germinator at a constant temperature of 30 C under continuous light. The control was not dried. Nine tubers were taken out of each fifth replicate to determine respiration rates.

Respiration rates were carried out by the Warburg Direct Method (62). Three tubers per flask were placed in 2 ml of deionized water. Two-tenths ml of 10% KOH was placed in the inner well of the flask. Respiration rates were then taken for 60 min.

Results and Discussion

This drying experiment was designed to determine the amount of drying that would inhibit hydrilla tuber sprouting after complete rehydration (Table 14). Water loss from the tubers was slow for the first 2 h of drying; after this time, there was a gradual increase in the amount of water lost from the tubers. This gradual water loss continued for 58 h. The respiration rate generally remained steady, with variability occurring at 4, 20, and 58 h. At the 20-h drying period, respiration increased, but there was a drop in sprouting of tubers. At drying periods of 32, 44, and 58 h, where there were 38 to 50% moisture losses, and respiration was erratic, the sprouting was reduced to zero. Generally, sprouting decreased with drying time (Figure 9). The 32-, 44-, and 58-h tuber samples were subjected to rehydration. The 32-h drying sample returned to 99% of its original weight, the 44-h sample to 95%, and the 58-h sample to 94% of its original fresh weight.

Figure 9. Sprouting of *Hydrilla verticillata* (L.f.) Royle tubers after several drying periods.

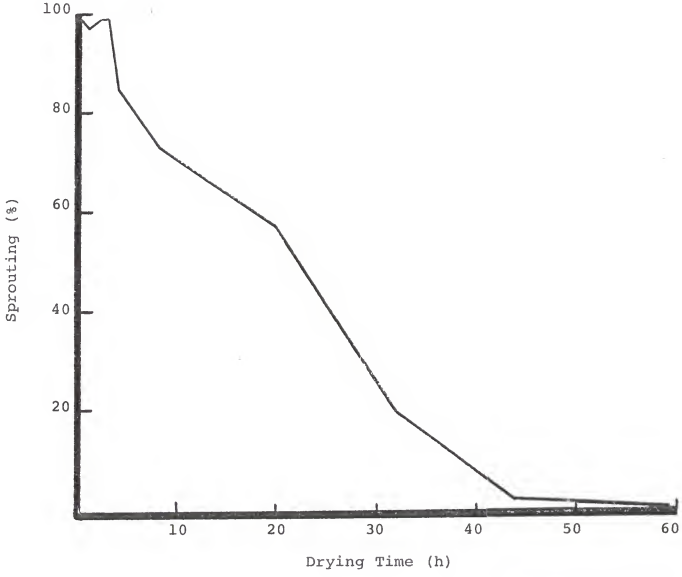

100
80
60
40
20
Sprouting (%)
10
20
30
40
50
60
Drying Time (h)

Table 14. Sprouting and respiration of hydrilla tubers after several drying periods.

Drying time (h)	Water loss (%)	Respiration rate (µl O_2/hr/g)	Sprouting after 3 days† (%)	Rehydration (%)
0		57	72	
1	2.36	46	64	
2	2.32	44	76	
3	7.49	41	78	
4	19.11	9	54	
8	22.01	45	48	
20	27.80	64	18	
32	38.80	37	0	99
44	50.62	48	0	95
58	47.09	87	0	94

†Each value is a percent of 50 tubers.

CHAPTER VIII
EFFECTS OF SEVERAL GROWTH REGULATORS AND HERBICIDES ON THE SPROUTING OF HYDRILLA TUBERS

Introduction

Efforts to control the infestation and spread of hydrilla by the use of herbicides have been futile. Tuberization occurs approximately 0.1 m below the hydrosoil. When the tuber reaches maturity, it becomes detached from the parent plant. Once the tuber becomes detached, it could go through a period of dormancy that could last for many months, depending upon the temperature and moisture of the immediate environment. The purpose of this study was to test the effects of several growth regulators and herbicides on the sprouting of hydrilla tubers.

Materials and Methods

Tubers collected from Rodman Reservoir during the month of May, 1975, were incubated in the herbicides banvel (3,6-dichloro-o-anisic acid), eptam (S-ethyl dipropylthiocarbamate), ethrel (2-chloroethylphosphonic acid), or endothall (the mono[N,N-dimethylalkylamine]salt of 7-oxabicyclo[2,2,1] heptane-2,3-dicarboxylic acid). Each herbicide was used at four different concentrations. For each concentration, four replicates of 10 tubers each were placed in Petri dishes. The replicates were then placed in a germinator under con-

tinuous light at 30 C. The tubers remained in the germinator for a period of 7 days.

The growth regulators gibberellic acid, thiourea, napthaleneacetic acid, and maleic hydrazide were used at one concentration. The tubers, along with the growth regulator, were placed in Petri dishes and allowed to remain in a germinator under continuous light at 30 C for a period of 7 days.

Results and Discussion

Banvel

Banvel in the dimethylamine salt form is soluble in water at a rate of 72 g/100 ml at 25 C. This herbicide is stable with regard to oxidation and hydrolysis. It is also resistant to acid and strong alkali. Banvel is mainly used as a preemergence to control annual braodleaf and grass weeds (21). The rates recommended for use are 0.56 to 3.36 kg/ha (21).

Banvel was applied to hydrilla tubers at rates of 0.29, 1.47, 2.94, and 5.88 mg/l. The sprouting percentages for the tubers were 98, 90, 98, and 98%, respectively (Table 15). Lengths of shoots were taken for each concentration. The shoots were generally short. The leaves appeared pale, as if the tubers had been sprouted in the dark. Many of the tubers started to decay within 4 days.

Eptam

Eptam is a selective herbicide for monocotyledons and some broadleaf weeds. It is frequently used in irrigation

Table 15. Some effects of herbicides on hydrilla tuber sprouting and shoot length.

Herbicide	Concentration mg/l	Sprouting† (%)	Meristem length†† (cm)
Banvel	0.29	98	2.29†††
	1.47	90	2.41†††
	2.94	96	2.51†††
	5.88	98	Decay
Eptam	0.58	98	2.06
	2.94	98	1.93
	5.88	98	1.87
	11.76	100	1.70
Ethrel	10.00	100	3.37
	50.00	98	3.96
	100.00	100	3.60
	200.00	100	2.49
Endothall	0.02	80	1.96
	0.11	93	1.81
	0.22	83	1.50
	0.43	95	1.20
Control	0.00	95	2.09

†Each percentage was for a sample of 40 tubers.

††Each meristem length is the average of 40 tubers.

†††Only two replicates could be used; the remainder decayed.

water. Eptam has a solubility in water of 370 mg/l at 20 C. The recommended rates of application are 2.74 to 6.73 kg/ha. Eptam translocates from the roots to stems and leaves. The mode of action of this compound is not known; however, a few mg/l have been found to inhibit growth in the meristematic region of grass leaves. Eptam is rapidly metabolized by plants to carbon dioxide and naturally occurring plant constituents. The breakdown of eptam in the soil is by microbial action (21).

Eptam was applied to hydrilla tubers at rates 0.58, 2.94, 5.88, and 11.76 mg/l. All of the concentrations used had sprouting percentages of 98 to 100. The shoot length decreased gradually with increases in herbicide concentration. Tubers sprouted in eptam appeared healthy and had very short sprouts after 4 days of incubation.

Ethrel

Ethrel is very soluble in water, and it causes ethylene to be released in plants. Ethrel is very mobile in plants and is metabolized by plants to naturally occurring substances. Methods and rates of application of ethrel vary depending upon the desired response in the plant.

Ethrel was applied to mature hydrilla tubers at rates of 10.06, 50.00, 100.00, and 200.00 mg/l. All of the samples had sprouting of 98 to 100%. The shoots produced were longer than those produced under any of the other herbicide treatments. The lengths of the shoots were 3.4 cm at application rates of 10.00 mg/l, 4 cm at 50.00 mg/l, 4 cm at 100.00 mg/l, and 2.5 cm at 200.00 mg/l. All of the tubers incubated in ethrel looked good after 4 days.

Endothall

This compound is stable to approximately 90 C. It is stable in light and in acid and soluble in water at the rate of 10 g/100 ml of water at 20 C. Endothall is an aquatic herbicide that is selectively toxic to plants. It is mainly used as a preemergence and as a postemergence. Endothall is broken down in soil and water by microbial action (21).

Endothall was applied to hydrilla tubers at rates of 0.02, 0.11, 0.22, and 0.43 mg/l. Tubers incubated at these concentrations had sprouting of 80, 93, 83, and 95%, respectively. The shoots were measured after 7 days. Tubers incubated at 0.02 mg/l endothall had shoot lengths of 1.96 cm. Those incubated at 0.11 mg/l had lengths of 1.81 cm, and those incubated at 0.22 and 0.43 mg/l had shoots 1.50 and 1.20 cm in length, respectively.

Effects of Several Growth Regulators on Hydrilla Sprouting

The growth regulators gibberellic acid, napthaleneacetic acid and thiourea had no negative effects on tuber-sprouting percentage (Table 16). Gibberellic acid and napthaleneacetic acid treatments permitted sprouting that ranged from 95 to 100%. Tubers placed in 500 mg/l maleic hydrazide had a sprouting percent of 73.

The lengths of the shoots were taken after 4 days. Tubers left in gibberellic acid had the longest shoots at 3.16 cm. Tubers in napthaleneacetic acid started to decay before measurements could be taken. Thiourea-treatment tubers had shoot lengths of 1.84 cm, and the control (water) tubers had lengths

Table 16. Some effects of growth regulators on hydrilla tuber sprouting and meristem length.

Growth regulator	Concentration (mg/l)	Sprouting (%)	Shoot length (cm)
Gibberellic Acid	1000	100	3.16
Maleic Hydrazide	500	73	0.50
Napthalene-acetic Acid	19	95	Decayed
Thiourea	761	100	1.84
Control (water)	0	95	2.09

Note: Values are the result of four replicates of 10 tubers each.

of 2.09 cm. All of the regulators permitted sprouting percentages similar to those allowed by the control except for maleic hydrazide. Tubers incubated in this compound showed marked differences in sprouting and in length of the shoots.

CHAPTER IX
SUMMARY AND CONCLUSIONS

1. The presence of hydrilla tubers in the Rodman Reservoir year round indicated that tubers did go through a period of dormancy. The length of the dormancy was not defined, since there was a large amount of variation among tubers. The variation among tubers was prevalent even under controlled conditions. Some of the tubers in shallow areas sprouted during the summer months, when the temperature of the water column was most favorable. Tubers formed at greater depths were still abundant and ready to sprout when conditions, either internal or external, became favorable.

2. Tubers were formed approximately 8 to 10 cm below the hydrosoil. The tubers were formed at the end of stolons. Upon reaching maturity, the newly formed tubers became detached from the parent plant.

3. The quantity and size of tubers in a particular area varied, depending upon the depth of the water column. There was also a large variation in the number of tubers within a small area.

4. Hydrilla tubers showed an increase in size with increase in depth of the water column from 0.3 to 1.5 m. The number of

tubers/m^2 increased with increase in water column depth from 0.3 to 0.9 m.

5. Tubers that were stored in moist peat under darkness at 25 C remained dormant for more than 9 months.

6. Calcium, potassium, magnesium, and iron were the most abundant mineral nutrients found in hydrilla tubers. Starch was the most abundant storage material. Crude protein, lipids, sucrose, reducing sugars, and nitrogen were present in lesser amounts.

7. Tubers sprouted under continuous light conditions consistently had higher sprouting percentages than tubers sprouted in darkness. This was true under both aerobic and anaerobic conditions. Tubers sprouted under light regimes of red, blue, green, and white light all had sprouting percentages of 95 to 100.

8. At low temperatures, tuber sprouting was inhibited. This inhibition was reversed when the tubers were placed under optimum sprouting conditions. The optimum sprouting temperature for hydrilla tubers was between 18 C and 32 C under dark conditions and 28 C under light conditions.

9. Tubers appear to need a state of anoxia in order to sprout. This was accomplished by covering the tubers with water.

10. Hydrilla tubers stored under 100% nitrogen for 14 days had good sprouting percent under either continuous light or dark conditions.

11. The storage of tubers under 100% oxygen for 14 days had little effect on sprouting, but when the tubers were stored in the dark, the sprouting percent was sharply reduced. Light appeared to stimulate sprouting when tubers were stored under oxygen.

12. The storage of tubers under 100% carbon dioxide for 14 days resulted in complete inhibition of tuber sprouting, even after the tubers were removed from carbon dioxide and placed under favorable sprouting conditions.

13. The time of storage, along with the percent carbon dioxide, appeared to affect sprouting rate. When the tubers were placed under favorable conditions for sprouting, this inhibition only lasted for a short while.

14. When tubers were stored for a month in sealed flasks, zero and 20% carbon dioxide had little effect on tuber sprouting. At 40, 60, 80, and 100% levels of carbon dioxide, the sprouting dropped off sharply. After tubers were removed from carbon dioxide and placed in Petri dishes, there was no increase in sprouting.

15. When tubers were dried for several time periods, the sprouting tended to decrease with increase in drying time. Tubers that were dehydrated for 1-2 h had small changes in moisture loss of 2.36 and 2.32%, respectively. After 3-h drying, there was a 7.49% moisture loss. The moisture loss at 4 h was 19.11% and the loss at 8 h was 22.01%. At 20, 32,

44, and 58 h dehydration, the percentages of moisture lost were 27.80, 38.80, 50.62, and 47.09, respectively.

16. Herbicides tested had little affect on sprouting percentages. They were effective after the tubers sprouted. Maleic hydrazide was the only growth regulator to markedly decrease sprouting and shoot length. Gibberellic acid, napthalene-acetic acid, and thiourea permitted 100, 95, and 100% sprouting, respectively.

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BIOGRAPHICAL SKETCH

James Lee Miller was born September 14, 1940, in Kershaw County, Kershaw, South Carolina. He graduated from Jackson High School, Camden, South Carolina in June, 1959. In September, 1959, he entered Voorhees School and Junior College, Denmark, South Carolina. Upon completion of his studies, he received a diploma in Liberal Arts.

After working one year for the United States Postal Service, he attended Tuskegee Institute, Tuskegee Institute, Alabama, receiving a Bachelor of Science in Biology in 1965 and Master of Science in Biology in 1968.

While at Tuskegee Institute he was a member of the Reserve Officers Training Program. In 1966, he was commissioned a Second Lieutenant in the United States Army Medical Service Corp. His duty stations included The United States Army Medical Training Center, Fort Sam Houston, Texas, where he served as training officer, executive officer, and company commander. The next duty assignment was with the First Air Calvary Division, Tay Ninh, South Vietnam, where he served as a battalion medical service officer. While in South Vietnam, he was wounded in action and received the Purple Heart. Upon returning to the United States, he was assigned to Fort Benning, Georgia, where he was a patient and also

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In June, 1971, he entered the University of Florida to pursue the degree Doctor of Philosophy in Agronomy. He is a member of the Omega Psi Phi Fraternity, The Weed Science Society of America, the American Society of Plant Physiologists, and the Hyacinth Control Society.

James L. Miller is married to the former Virdell McNair of Fort Lauderdale, Florida. They have two children, Pamela and James.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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